



Tumor gene therapy by MVA-mediated expression of T-cell-stimulating antibodies

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Immune responses to tumor-associated antigens are often dampened by a tumor-induced state of immune anergy. Previous work has attempted to overcome tumor-induced T-cell anergy by the direct injection of vectors carrying the genes encoding one of a variety of cytokines. We hypothesized that the polyclonal stimulation of T cells, preferably through the TCR complex, would result in a cascade of cytokines associated with T-cell activation and would be best able to overcome T-cell anergy. Here we use the highly attenuated MVA poxvirus to express on tumor cells, *in vitro* and *in vivo*, either of three membrane-bound monoclonal antibodies specific for murine TCR complex. Using this system, we have expressed antibodies specific for the CD3 ϵ chain (KT3), TCR α/β complex (H57-597), and V β 7 chain (TR310). Tumor cells bristling with these antibodies are capable of inducing murine T-cell proliferation and cytokine production. When injected into growing tumors (P815, RenCa, and B16F10), these constructs induce the activation of immune effector cells and result in the rejection of the tumor. Histological and FACS analysis of tumor-infiltrating leukocytes reveal that the injection of recombinant virus-expressing antibodies specific for the TCR complex attracts and activates (CD25⁺, CD69⁺) CD4 and CD8 lymphocytes. This approach represents a novel strategy to overcome T-cell anergy in tumors and allow the stimulation of tumor-specific T cells.

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Current efforts to develop immunotherapy of cancer focus on the induction of tumor-specific anamnestic immune response. Several strategies have been developed to recruit specific effector T cells towards tumors and to stimulate them locally. Bispecific antibodies with specificities for the CD3 molecule on T cells and tumor cell surface antigen can target effector T cells towards tumor cells.¹ Limitations with this approach are that the identity of the tumor antigen is required and that intravenous administration of bispecific antibodies can induce systemic cytokine release and toxicity.² The use of cytokines exhibiting pleiotropic activities has also been investigated in order to circumvent the immune anergy associated with many cancers and to promote a tumor-specific T-cell-mediated immune response.³ Because systemic delivery of high doses of cytokines is toxic, *in situ* expression of cytokine genes in solid tumors is considered preferable.⁴ However, therapeutic effects resulting from the overexpression of a single cytokine are often limited and depend upon natural killer or other nonantigen-specific cellular effectors.⁴

One interesting avenue is to use the cytokine cascade associated with strong antigenic stimulation of T cells. This can be achieved with monoclonal antibodies (mAbs)

recognizing certain T-cell surface molecules (i.e., TCR, CD3, CD2) that mimic antigenic stimulation of T cells.⁵ The relevance of this approach was demonstrated when tumor-bearing mice rejected their tumors following *in situ* injection of an anti-CD3 antibody.⁶ Also, tumor cells have been engineered to express anti-CD3 antibodies by *in vitro* transduction.^{7,8} Murine tumor cells engineered to express anti-CD3 antibodies prior to implantation induce a T-cell-mediated control of tumor growth.⁷

Materials and methods

Antibodies and cytokines

FITC-labeled or unconjugated mouse anti-rat IgG, rat anti-hamster IgG, and anti-rat kappa light chain were purchased from Jackson ImmunoResearch Laboratories (Pennsylvania, PA) and used for FACS or Western blot detection of TCR-specific mAbs. FITC or phycoerythrin-labeled rat anti-mouse CD4, CD8, CD3, CD69, MAC1, CD11c, and Ia^b or unconjugated rat anti-mouse CD4 and CD8 were used as defined by the manufacturer (Pharmingen, San Diego, CA). Unconjugated rabbit anti-human CD3 (which crossreacts with mouse CD3) or rabbit anti-rat IgG and peroxidase-labeled goat antirabbit were used at concentrations suggested by Dako (Trappes, France).

Recombinant human IL-2 was purchased from R&D Systems (Abingdon, UK). Concanavalin A (ConA) was

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used at 1 µg/mL and purchased from Sigma-Aldrich (Saint Quentin Fellavier, France).

Tumor cells. P815 murine mastocytoma (DBA/2; FcR⁺, H2D^d, MHCI⁺, ICAM1⁺, CD48⁺) and B16F10 (C57Bl/6; H2D^b, MHCI⁻, MHCII⁻, ICAM1⁻, CD48⁻) murine melanoma cancer cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA). RenCa murine renal carcinoma (BALB-C; H2D^d, MHCI⁺, MHCII⁺, Fas⁺) cell line was the generous gift of Dr. G Hillman (University of Chicago). BHK-21 cells were also provided by ATCC (Rockville, MD). All cell lines were tested negative for mycoplasma using Hoechst dye, cell culture, and polymerase chain reaction.

Hybridoma. TR310 (rat anti-mouse Vβ7; IgG2b) and H57-597 hybridomas (hamster anti-mouse TCRαβ; IgG) cell lines were obtained from the ATCC. KT3 hybridoma (rat anti-mouse CD3ε; IgG2a) was generously provided by Dr. D Mathis (Harvard Medical School, Boston, MA).

Recombinant viruses. Recombinant viruses were constructed as reported elsewhere.⁹ Briefly, the early-late vaccinia virus promoters pH5R¹⁰ and p7.5¹¹ were isolated from vaccinia virus WR DNA by polymerase chain reaction. Heavy- and light-chain cDNA of the antibodies were synthesized from total RNA extracted from KT3, H57-597, and TR310 hybridomas using the Universal Riboclone cDNA Synthesis System (Promega, Madison, WI) and cloned into a pBlue-

script vector for sequencing. The heavy chain of each mAb was cloned in phase upstream of the sequence coding for the transmembrane and intracytoplasmic domains of the rabies glycoprotein (Fig 1).^{12,13} The cDNA for the native light and modified heavy chains were introduced into MVA transfer plasmid vector downstream of 7.5 kDa or ph5r vaccinia promoters, in reverse orientations similar to that described previously.⁹

Generation of MVATG14205 (TR310), MVATG14237 (H57), and MVATG14240 (KT3) was performed by homologous recombination in primary chicken embryo fibroblasts using pTG14205, pTG14237, and pTG14240 MVA transfer plasmid (Fig 1).⁹ Purification of viral stocks generated viral suspensions devoid of recombinant mAbs (data not shown).

Detection of TCR-specific mAbs expression

Antibody expression was analysed by Western blot according to the ECLTM Western blotting protocol provided by Amersham Life Sciences (Amersham, UK). Primary chicken embryo fibroblasts were infected at a multiplicity of infection (MOI) of 1. Twenty-four hours after infection, the cells were washed once with PBS and disrupted in sample buffer (Novex) by sonication. Homogenates were heated for 3 minutes at 95°C prior to fractionation on a 13% SDS polyacrylamide gel. Fractioned proteins were transferred from the gel to a PVDF membrane. The TCR-specific

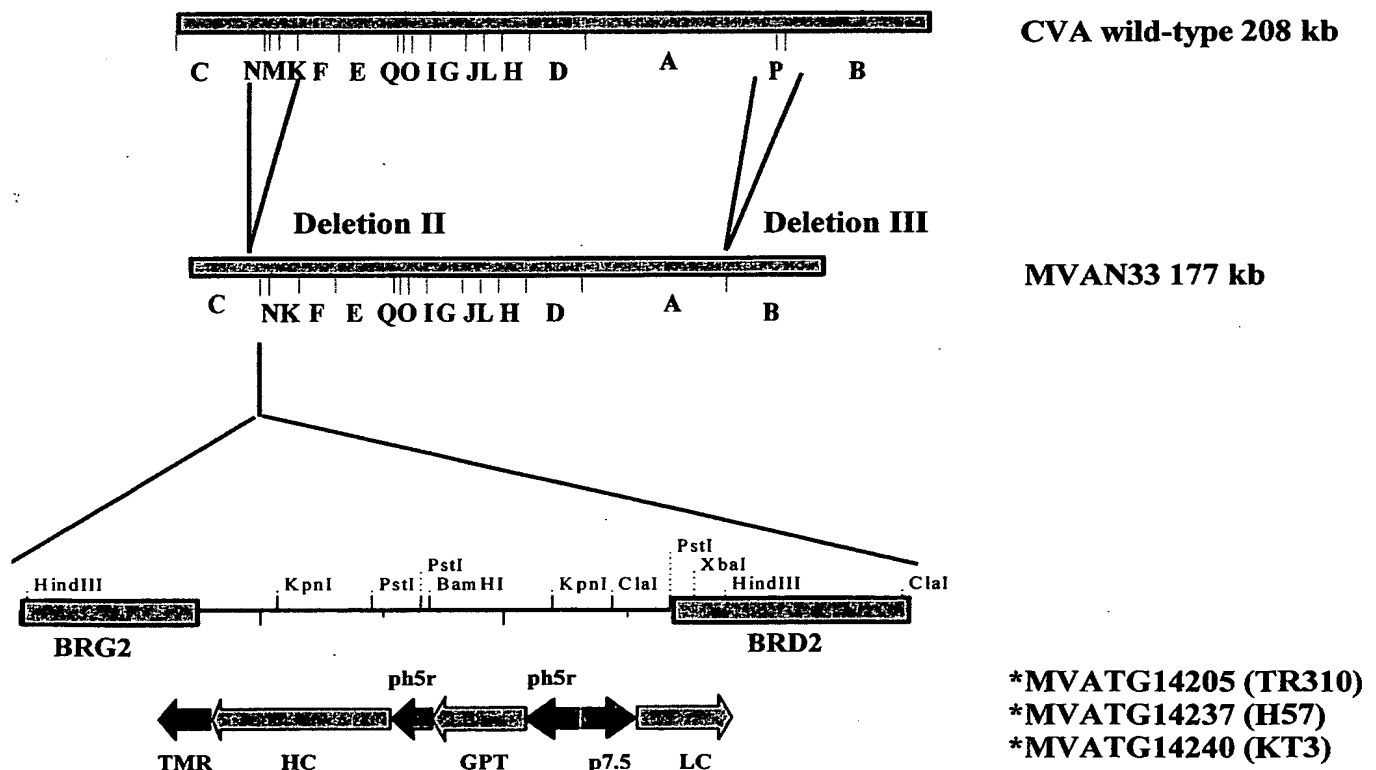


Figure 1 Schematic representation of recombinant MVA. CVA: parental Copenhagen virus A; BRG 2 and BRD2: flanking sequences surrounding deletion II of MVA; alphabetic characters correspond to *HindIII* fragments of MVA genome; ph5r: early-late vaccinia virus promoter; p7.5: early-late vaccinia virus promoter; GPT: *Escherichia coli* xanthine-guanine phosphoribosyltransferase selection gene; TMR: sequence coding for the transmembrane and intracytoplasmic domains of rabies glycoprotein; HC: heavy-chain cDNA; LC: light-chain cDNA.

mAbs were probed using biotinylated anti-IgG and revealed with horseradish peroxidase-labeled streptavidin (10 $\mu\text{g/mL}$).

TCR-specific mAbs expression in primary chicken embryo fibroblasts was also analysed by FACS using FITC-labeled goat anti-rat or mouse anti-hamster IgG.

MVA infection of cells for flow cytometry analysis. BHK-21 cells were infected overnight at an MOI of 0.1. The following day, cells were washed and assessed for expression of membrane bound antibody as described elsewhere.⁹

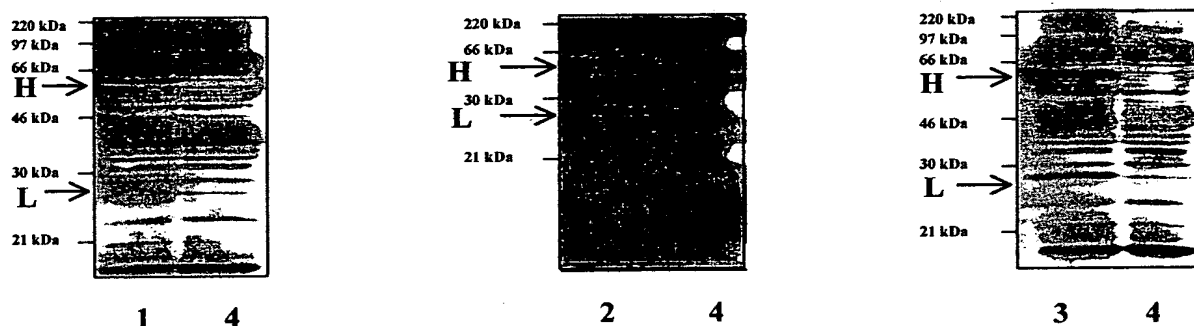
T-cell proliferation assay. Mouse spleen cell proliferation was assessed by the uptake of [^3H]thymidine. Target tumor cells (RenCa or B16F10) were infected at an MOI of 0.5 for 12 hours then treated with mitomycin C (50 $\mu\text{g/mL}$; Sigma) for 1 hour. After washing, infected tumor cells and splenocytes were co-cultured at the ratios indicated. As positive control, spleen cells (5×10^4 cells/well) were stimulated in complete medium with either ConA (10 $\mu\text{g/mL}$) or 100 ng/mL recombinant murine IL-2 (R&D Systems). After 96 hours, the cells were pulsed with 1 μCi /well [^3H]thymidine. Incorporation of [^3H]thymidine into the DNA of proliferating T cells was measured by harvesting cellular DNA onto glass filter paper (PHD Harvester; Cambridge Technology, Cambridge, UK) after 4 hours and

by counting the radioactivity in a liquid scintillation counter (Beckman Coulter, Roissy, France). All measurements were in triplicate.

Measurement of supernatant cytokine content. P815 cells were infected at an MOI of 0.5 for 12 hours then treated with mitomycin C (50 $\mu\text{g/mL}$; Sigma) for 1 hour. After washing, infected tumor cells and splenocytes were co-cultured at a 1:10 ratio. As positive control, spleen cells (5×10^4 cells/well) were stimulated in complete medium with ConA (10 $\mu\text{g/mL}$). As negative control, spleen cells were co-cultured for 24 hours with P815 cells infected with MVAN33 or preincubated with an irrelevant isotype-matched antibody (50 $\mu\text{g/mL}$). Culture supernatants were then tested for IL-4 or IFN- γ content using specific immunoassay (Quantikine; R&D Systems) according to the manufacture's instructions. All measurements were in triplicate.

In vivo experiments. The mouse P815, B16F10, and RenCa tumors were established by injecting 100 μL of PBS containing 3×10^5 cells subcutaneously into the flanks of 6-week-old female immunocompetent B6D2 F1 mice (IFFA CREDO, Lyon, France). Eight days later, when tumors were palpable, the mice were randomized (10 mice per group) and their tumors were injected with the indicated MVA vectors as follows. Viruses were resuspended in 10 mM

A



B

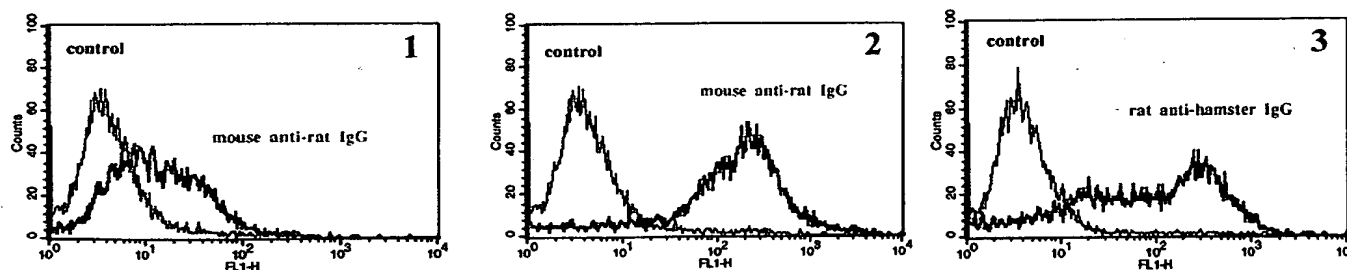


Figure 2 *In vitro* functionality of MVA recombinants expressing TCR/CD3-specific antibodies. **A:** Western blot analysis of extracts of BHK-21 cells infected with MVA-TR310 (1), MVA-H57 (2), MVA-KT3 (3), or with control virus (MVAN33, 4). Molecular weight markers are indicated on the left. Heavy (H) and light (L) chains are indicated. **B:** Flow cytometry analysis of TCR/CD3-specific antibodies expression on BHK-21 infected by MVA-TR310 (1), MVA-KT3 (2), or MVA-H57 (3).

Tris-HCl, pH 7.5, 10 mM MgCl₂ at a concentration of 2×10^7 pfu/100 μ L, and injected into the tumors on days 8, 10, and 12. Tumor sizes were measured using calipers. Tumor volumes were calculated in cubic millimeter using the formula: $(\pi/6)(\text{length} \times \text{width} \times \text{height})$.

Statistical analyses. Statistical analyses were performed using the log rank application for Kaplan-Meier survival curves and the *U* test of Mann-Whitney for tumor volume curves (Statistica 5.1 software; StatSoft). A *P* < .05 was considered statistically significant.

Immunohistochemistry or flow cytometry analysis of in vivo response. Tumors were established and injected with the various viruses as described above for *in vivo* experiments. On day 13, tumors were measured and excised. For flow cytometry analysis, tumors were disrupted by collagenase (Sigma) digestion, cells were stained with the indicated antibodies, and population analysed by cytofluorimetry.¹⁴ For immunohistochemistry, tumors were fixed in 10% neutral formalin. The samples were embedded and sectioned to 5 μ m. After rinsing and fixing with acetone, slides

were incubated with primary antibodies. After washing, slides were incubated with the secondary rabbit anti-rat immunoglobulin. Slides were then rinsed and incubated with a peroxidase-conjugated goat anti-rabbit Ab for amplification of the response, followed by DAB (diaminobenzidine) color development. Slides were observed at $\times 20$ magnification.

Results

Construction of MVA expressing T-cell-stimulating antibodies

We have used the highly attenuated Modified Ankara Virus (MVA) to deliver and express in tumor cells membrane-bound mAbs specific for murine CD3 ϵ chain (KT3),¹⁵ TCR α/β complex (H57-597),¹⁶ or V β 7 chain (TR310).¹⁷ In each case, the heavy chain of the antibody is fused, as shown in Figure 1, to the membrane anchoring region of the rabies virus glycoprotein, which has been shown to remain attached to the surface of infected cells.¹⁸

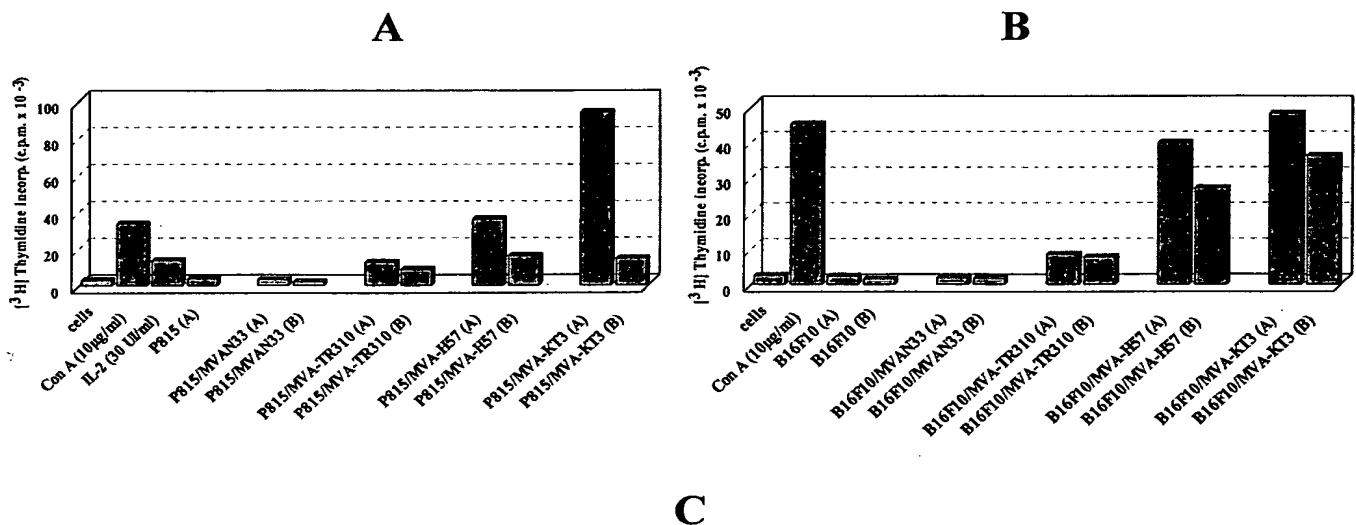


Figure 3 A,B: *In vitro* T-cell proliferation induced by tumor cells [P815(C); B16F10(D)] infected with the recombinant MVA. As positive control, splenocytes were stimulated with 10 μ g/mL ConA or 100 ng/mL rIL-2. As negative control, tumor cells or control virus-infected tumor cells were used. For each construct, two concentrations of infected tumor cells were evaluated (A = 10,000 cells/well or B = 1000 cells/well) with a constant number (1×10^5) of splenocytes per well. Similar results were found in repeat experiments. Standard deviation never exceeded 5% of reported values (not shown). **C:** Measurement of mouse IL-4 or IFN- γ in the supernatants from co-cultures of splenocytes and infected P815 cells (MVA-TR310, MVA-H57, and MVA-KT3). As positive control, spleen cells were stimulated in complete medium with ConA. As negative controls, spleen cells were co-cultured with P815 cells infected with MVAN33 or preincubated with an irrelevant isotype-matched antibody (Ab). Results are expressed as picograms per milliliter per million effector cells per 24 hours.

All MVA recombinants were shown to infect tumor cells *in vitro*, and to promote the expression of mAbs of the correct specificity on the tumor cells (Fig 2, A and B). For MVA-TR310, the band corresponding to the kappa light chain is faint but the expression is also detectable by flow cytometry analysis (data not shown). Cells infected with these constructs, but not with the negative control construct (MVAN33), expressed antibody heavy and light chains that remained membrane-anchored (data not shown). Previous reports have shown that mAbs specific for the T-cell receptor complex were able to stimulate T cells when bound to a matrix and able to crosslink the TCR complex. This includes binding to beads, to plastic tissue culture vessels, or on antigen-presenting cells through the Fc receptor.^{19,20} In contrast, high doses of soluble anti-CD3 (OKT3) are immunosuppressive.^{21,22}

In vitro immune stimulation

We first showed that Fc receptor-positive (FcR⁺) P815 murine tumor cells coated with exogenously added purified mAbs (KT3, H57, and TR310) can be used as a matrix to

stimulate *in vitro* T-cell proliferation (data not shown). P815 and B16F10 tumor models were then chosen because of their distinct immunogenicity and genetic background, and were infected with the three MVA recombinants or control MVA. The ability of antibody-expressing cells to induce proliferation of autologous murine spleen cells is clearly shown in Figure 3, A and B). The construct carrying the heavy and light chains for the TR310 mAb (MVA-TR310) was the least effective (Fig 3, A and D). This can be explained by the fact that only 3% of the entire T-cell repertoire in DBA or C57Bl/6 expresses T-cell receptors made with the V β 7 variable region.¹⁷ The following experiments were restricted to the anti-TCR α/β mAb (MVA-H57) or the CD3 ϵ -specific mAb (MVA-KT3) constructs. Measurement of IL-4 and IFN- γ content of supernatants obtained after *in vitro* immune stimulation indicates evidence of the induction of both Th1 and Th2 T-cell stimulation with both MVA-H57 and MVA-KT3 (Fig 3C).

Effects on in vivo tumor growth

The consequences of intratumoral expression of either H57 or KT3 on tumor growth and mouse survival in three murine

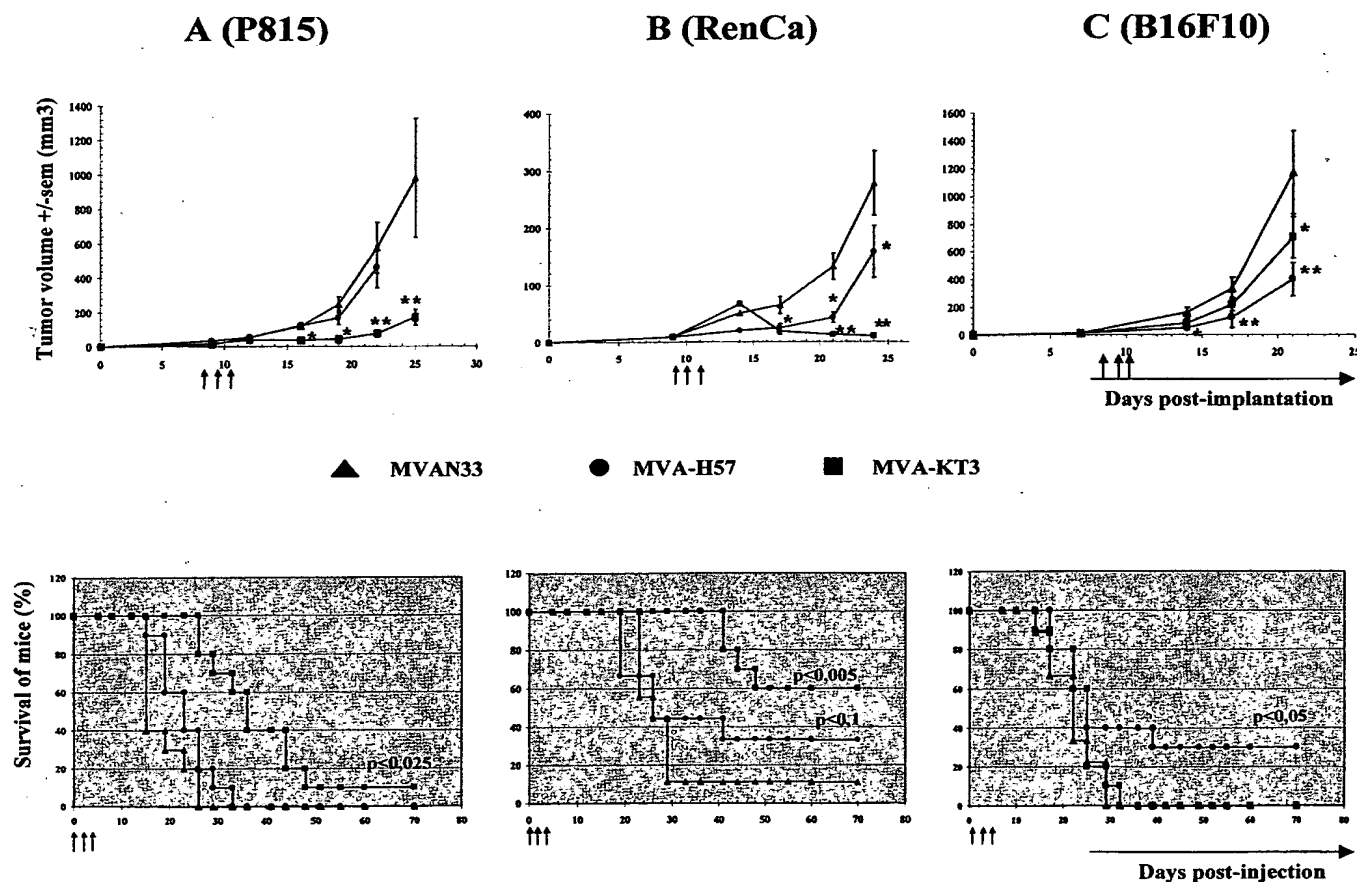


Figure 4 *In vivo* anti-tumor activity of MVA expressing TCR-specific antibodies using P815 (A), RenCa (B), and B16F10 (C). Tumors growing in B6D2 mice (groups of 10) were injected three times with 2×10^7 pfu of control MVAN33 (▲), MVA-H57 (●), or MVA-KT3 (■). Tumor volumes (mm³) and survival of mice (%) were monitored. Significant differences between the mean survival of animals treated with control virus or recombinant MVA are indicated. Statistical values were calculated as described in Materials and Methods and are indicated in the figure: * $P < .05$; ** $P < .005$. Vertical arrows indicate days of virus injection.

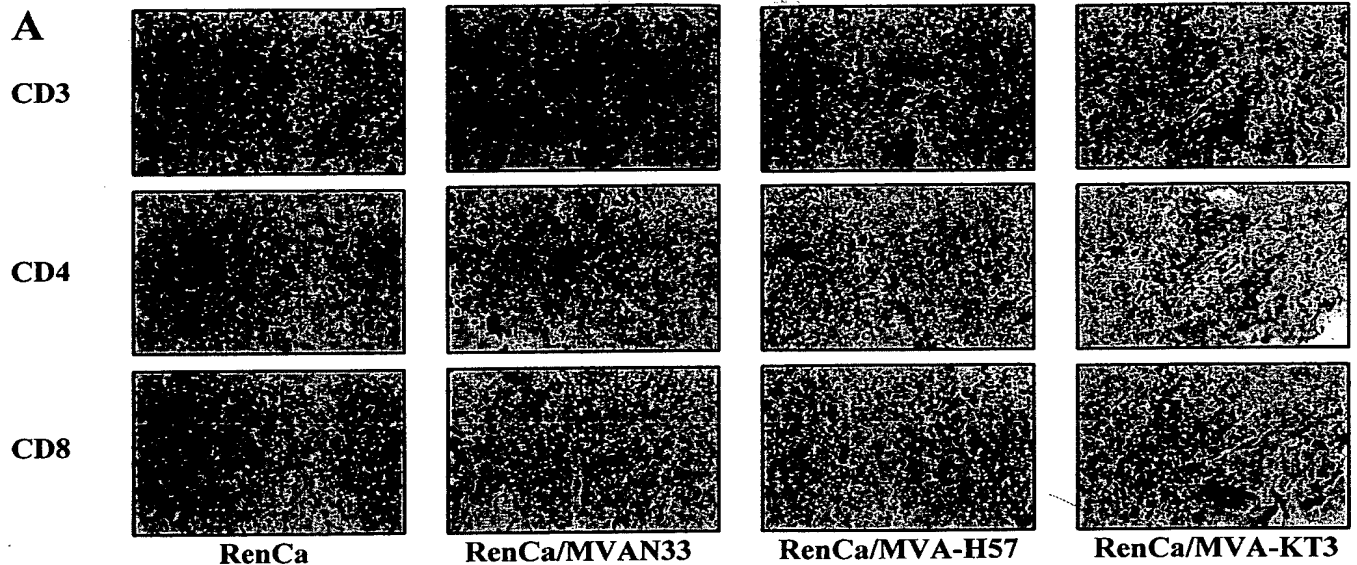
Table 1 Tumor-specific immunity was evaluated in surviving mice after MVA-H57 or MVA-KT3 treatment, by reinjecting the same tumor cells (B16F10 or RenCa) contralaterally 70 days postinitial implantation

	B16F10	RenCa
MVA-H57	3/3 (100%) 4/4 (100%)	1/3 (33%) 0/3 (0%)
MVA-KT3	0/0 0/0	6/6 (100%) 7/7 (100%)

Survival of mice (%) was monitored.

tumor models (P815, RenCa, and B16F10) in B6D2 mice were investigated. *In vitro* experiments have shown that the infection efficiency with recombinant MVA is not the same with these three tumor models. RenCa and B16F10 infect at the same rate (50% at an MOI of 0.1), but P815 infects at a rate which is 10 times less (data not shown). Infection rates *in vivo* are all approximately the same (about 1% of tumor cells with an injection of 2×10^7 pfu).

Both recombinant vectors clearly influenced tumor growth in each model examined with the exception of MVA-H57 in the P815 model (Fig 4, A–C). This correlates with an increase in the survival time of treated mice. In the RenCa model, 60% and 33% of mice injected with MVA-KT3 and MVA-H57 completely rejected their tumors, respectively. In the B16F10 model, 30% of tumors was rejected when injected with MVA-H57, whereas MVA-KT3 was ineffective. One explanation for this difference could be that antibodies which crosslink the T-cell receptor (such as H57) induce a different signal transduction pathway in T cells than antibodies which crosslink the CD3 complex (as does KT3).²³ Nevertheless, it is unclear at present whether the observed difference in the efficacy of MVA-KT3 and MVA-H57 can be attributed to the nature of the signalling pathway, to inherent immunogenicity of the antibodies (rat *versus* hamster antibody), to differences in cell surface antigen expression between B16F10 and RenCa cells, or to infection and/or expression capacity of the tumor cells. The construct MVA-TR310 was also tested for tumor immunotherapy in both the P815 and RenCa

**B**

Infiltrating Cells (%)	RenCa	MVAN33	MVA-H57	MVA-KT3
CD4+	0 ± 0	4,2 ± 2	9 ± 3	12 ± 2
CD4+/CD25+	0 ± 0	0,7 ± 0,3	3,8 ± 1	6,2 ± 0,4
CD8+	4 ± 1	11 ± 1	17 ± 3	33 ± 5
CD8+/CD25+	0,2 ± 0,01	0,9 ± 0,1	4,5 ± 1,5	14,5 ± 3
CD3+	4 ± 0,2	12,8 ± 0,2	28,6 ± 2,5	40 ± 5
CD3+/CD25+	0,04 ± 0,01	2,4 ± 0,3	12 ± 0,5	17,5 ± 3
CD3+/CD69+	0,1 ± 0,01	2,5 ± 0,1	13,2 ± 3	22,9 ± 0,8
MAC1+	11 ± 2	30 ± 5	35 ± 4	37,6 ± 2
CD11c+	1 ± 0,1	6 ± 2	6,7 ± 0,3	19 ± 2

Figure 5 Tumor immune cells infiltration induced by recombinant MVA expressing TCR complex - specific antibodies injection in RenCa tumors. **A:** Tissue sections were prepared and stained as described (Materials and methods). **B:** FACScan analysis of single cells suspension prepared from the tumors and stained as described (Materials and methods). Percentages represent the number of positive cells in 1×10^5 ungated total events. Each result is representative of three animals, each with similar results. Standard deviations are indicated.

models. In both of these model systems, this construct was ineffective, indicating that the inherent immunogenicity of a membrane-bound rat antibody is not sufficient to induce rejection of the tumor (data not shown). All experiments included a group whose tumors were injected with buffer alone. The survival curves of these animals did not differ significantly from that of the groups injected with the negative control virus MVAN33 (data not shown).

Tumor-specific immunity was evaluated in surviving mice by reinjecting the same tumor cells contralaterally 70 days postinitial implantation. Mice treated with MVA-KT3 and having rejected RenCa tumors were all resistant to the second challenge (Table 1). In contrast, only one of three surviving mice treated with MVA-H57 could reject the second RenCa tumor challenge (Table 1). In the B16F10 model, all mice surviving following the MVA-H57 treatment were protected against rechallenge (Table 1). This is in contrast with previously reported experiments in which *in vitro* transfected tumor cells expressing membrane bound anti-CD3 antibodies prior to implantation resulted in control of tumor growth and did not allow the establishment of a tumor-specific immune memory.⁷

Phenotype analysis of intratumor leukocytes

In vivo growing RenCa tumors injected with the MVA recombinants were examined for immune cell infiltration by immunohistochemistry at day 5 postvirus injection. Immunohistochemistry analysis (Fig 5A) demonstrated that injection of control MVAN33 attracted some T cells to the tumor, but these were restricted to areas around tumor vascularisation (data not shown). In contrast, tumors injected with MVA-H57 and MVA-KT3 were shown to be infiltrated with both CD4- and CD8-expressing T cells. Cytofluorimetry analysis of extracted tumors showed that these T cells expressed CD25 and CD69, both of which are markers of T-cell activation (Fig 5B). Treatment with either recombinant virus also attracted a greater number of the CD11c⁺ dendritic cells found to be mature (CD11c⁺/Ia^{b+}) in MVA-KT3-treated tumors. It is noteworthy that MVA-KT3 injected into RenCa tumors attracted more infiltrating cells than MVA-H57, which correlates well with their respective efficacy on tumor growth and animal survival, as shown in Figure 4B.

Discussion

In this report, we have described a novel method for stimulating T cells *in situ* by using recombinant MVA expressing membrane-bound T-cell-stimulating mAbs. By applying this approach to models of immunotherapy of solid tumors, we have demonstrated that it results in the attraction of T cells to the tumor site, some of which have an activated phenotype. This is in contrast to observations using adenoviral vectors expressing single cytokines (IL-2, IL-12) in which lymphocytes are attracted into the tumor but show no signs of activation (personal observations). The experimental results showing that tumors were rejected upon rechallenge suggest that infiltrating leukocytes could contain tumor-specific effector cells. In addition, mice who died

from their tumors were observed to have multiple metastasis in their internal organs (lungs, liver, and spleen). Mice whose tumors had been rejected after vector administration showed no evidence of tumor metastasis upon sacrifice.

Importantly, this approach differs from those using soluble Abs (such as soluble anti-CD3 or bispecific Abs) therapy in several ways. In those applications, CD3⁺ cells are immediately coated with antibody after systemic administration of specific or bispecific Abs, causing the Ab-coated lymphocytes to leave the circulation and traffic to lymphoid organs.²⁴ Another limitation of bispecific antibodies is that antibody-coated lymphocytes exhibit only one cytolytic cycle, requiring the administration of excess antibody or infusion of large numbers of functional T cells with specific antitumor reactivity into patients in order to achieve good therapeutic efficacy. Moreover, purified antibodies are rapidly diluted upon injection and are likely to exhibit a very short half-life.²⁵ T-cell activation by TCR- or CD3-specific, membrane-anchored antibodies, as expressed by the MVA vectors, is expected to be limited to tumor-infiltrating lymphocytes and thus is likely to reduce the potential toxicity of the treatment and the immunogenicity of the antibody.

T cells typically require two signals for activation — one produced by interaction of the TCR with its specific ligand, and the other generated by the interaction of co-stimulatory molecules such as CD80 or CD86 on APCs with CD28 on T cells. Whether the therapeutic effect of this approach can be increased by co-expression of these or other T-cell co-stimulatory molecules is currently under investigation.

There are several fundamental differences between RenCa and B16F10 that could account for the observed difference in rejection frequencies upon injection with the MVA-KT3 construct. B16F10 tumors grow much more quickly than do RenCa tumors, which may result in outgrowth of the tumor prior to an effective immune response. RenCa cells express MHCI, whereas B16F10 express little or no MHCI.²⁶ Growth of RenCa tumors has been shown to involve T-cell-mediated immune suppression.²⁷⁻²⁹ RenCa cells express ICAM-1, whereas B16F10 cells do not express ICAM-1 (our own unpublished data) and rejection of the tumor has been observed to depend upon expression of ICAM-1.³⁰ These, as well as other possible differences between RenCa and B16F10 tumors, may account for the differences observed in effectiveness of MVA-KT3 in the control of tumor growth.

It has been shown that stimulation of T cells through the CD3 complex can differ from T-cell stimulation through the TCR itself.²³ Therefore, despite the ability of both to stimulate IL4 and IFN γ equivalently, there may well be other cytokines or activities stimulated by the anti-TCR construct which are not, or less efficiently, stimulated by the anti-CD3 construct and *vice versa*. In view of the numerous differences between B16F10 and RenCa, as described above, this could have an impact on therapeutic effects of these two viral constructs as well as observed differences in the ability to induce effective immune memory.

It has been reported recently that the intravenous injection of humanized soluble anti-CD3 antibody into cancer patients resulted in the resolution of malignant ascites in 3

of 24 patients.³¹ This observation, together with our own results, suggests that the expression of TCR-specific antibodies using an MVA vector may form the basis of a novel tumor therapy strategy especially suited for accessible tumors exhibiting various immune escape mechanisms.³²

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